In order to measure the noise of promoters, we needed to ensure that each cell only contained one copy of each promoter. To do this, we integrated our fluorescent constructs onto the *E. coli* genome.

In order to integrate, we followed protocols described by previous iGEM teams: Paris-Bettencourt and Harvard. We used the EcNR2 strain of *E. coli*, obtained from Addgene, originally from the lab of Dr. George Church. This strain both contains necessary integration machinery under the control of a heat sensitive promoter, as well as has the gene mutS knocked out, in order to help increase the chances of successful integrations. It is chloramphenicol and ampicillin resistance.

We integrated in two different locations, referred to by us as *intC* and *galK*.

Location *intC* codes for a prophage integrate. Location *galK* is a gene that codes for galactokinase, a product that was deemed unessential. These are the locations that were used by Dr. Elowitz in his 2002 paper, *Stochastic gene expression in a single cell*. Although the regions we have selected are separated only by approximately 1,000 base pairs, we have shown that these regions work for inserts as long as approximately 2kB.

In order to integrate a part, it must be tethered to an antibiotic, so that we may select for cells that have had the construct of part+antibiotic successfully integrated. We did this in two ways:

1. We created antibiotic resistance operons for kanamycin resistance and tetracyclin resistance (see parts page). We assembled these operons with the parts that we wanted to integrate, and then PCR'd the assembly with the following integration primers:

intC forward:
5' CCGTAGATTTACAGTTCGTCATGGTTCGCTTCAGATCGTT
GACAGCCGCAATAAAAAAAATCCTTAGCTT 3'

intC reverse:
5' ATAGTTGTTAAGGTCGCTCACTCCACCTTCTCATCAAGCC
AGTCCGCCCAGTGGTGACACCTTGCCCTTT 3'

galK forward:
5' TTCATATTGTTCAGCGACAGCTTGCTGTACGGCAGGCACC
AGCTCTTCCGATAAAAAAAATCCTTAGCTT 3'

galK reverse:
5' GTTTGCGCGCAGTCAGCGATATCCATTTTCGCGAATCCGG
AGTGTAAGAAGTGGTGACACCTTGCCCTTT 3'

The section of the primer highlighted in yellow is the section that is homologous to either the intC region or galK region in the *E. coli* genome. The unhighlighted section will bind to the prefix/suffix, and will amplify up the assembly product of part-to-integrate+antibiotic resistance.

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2. We created a galK integration cassette, an altered backbone that contains a prefix, suffix, and an antibiotic resistance between the homologous regions. These homologous regions are exactly the same as those that are created by PCR with the galK primers.

In order to use the integration suite, we assembled our part to be integrated onto the suite with Gibson assembly, between the prefix and suffix (users may also insert their part onto the suite with 3A assembly). We then used the following primers to PCR up the plasmids containing the integrator suites:

galK forward:
5' TTCATATTGTTCAGCGACAG 3'

galK reverse:
5' GTTTGCGCGCAGTCAGCG 3'

Once we had the PCR products, either from method 1) or method 2), we performed a DpnI reaction, to remove residue template plasmid, and then PCR-Purified the digested products (QIAGEN QIAquick PCR Purification kit).

We used this product in the integration protocol described by Paris-Bettencourt and Harvard. Our overnight starter cultures of EcNR2, in preparation for electroporation, were grown up in Low Salt LB:

- 2.5 g NaCl
- 2.5 g yeast extract
- 5 g tryptone
- 500 ml water
- Autoclave

Genome Integration